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Brominated fatty acids from lichen *Acorospora gobiensis*

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Abstract

New brominated C18 acetylenic acids have been isolated from terrestrial lichen *Acorospora gobiensis*. Spectroscopic methods as well as the comparison of spectral data with those known related compounds determined their structures. Also the bromoperoxidase activity were identified. The enzyme contained vanadium. © 1998 Published by Elsevier Science Ltd. All rights reserved.

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1. Introduction

Brominated fatty acids are rare in nature (Grible, 1996). These acids, in particular those arising from marine invertebrates have received considerable attention during the last 15 years (Mu et al., 1997). Marine invertebrates are the only known sources of naturally occurring brominated fatty acids. Originally they were found in sponges (Mu, Wesen, & Sundin, 1997) and recently they were also reported in anemone (Carballeira & Reyes, 1995). The long chain fatty acids, i.e. (5*E*,9*Z*)-6-bromo-5,9-dienoic acids, were isolated from some sponges (Carballeira, 1997). Later also other brominated fatty acids have been documented in marine animals. They were predominantly unsaturated mono- and bisacetylenic, mono- and dibrominated straight chain C_{16}, C_{18} acids, but some other long chain fatty acids (Schmitz & Gopichand, 1978; Hirsh, Carmely, & Kashman, 1987; Quinn & Tucker, 1985; Quinn & Tucker, 1991; Bourguet-Kondracki, Rakotoarisoa, Martin, & Guyot, 1992; Patil et al., 1992; Ichiba, Scheuer, & Kellyborges, 1994; Brantley, Molinski, Preston, & DeLong, 1995) were also detected.

In earlier papers (Rezanka & Sokolov, 1990; Rezanka & Dembitsky, 1993; Rezanka, Dembitsky, & Kashin, 1994) we have described the isolation of many novel fatty acids with unique characteristics such as increased chain length, branching and unusual unsaturation. This paper continues in investigating in the area, this time on lichens from saline soils on the waterside of Lake Issyk-Kul from the Asian Tian-Shan Mountains. This paper is probably the first, which describes the presence of brominated fatty acids in terrestrial organism and, what is more, even in plants.

2. Results and discussion

Issyk-Kul is a large and brackish mountain lake, which is bordered on the north and south by the snow-clad chains of Central Asian Tian Shan Mountains. It is one of the largest mountain lakes in the world and one of the deepest lakes in Central Asia. The lake basin is of tectonic origin and it has probably existed since the end of the Paleozoic era. Thus, it is one of the oldest lakes in Central Asia (Berg, 1950; Matveyev, 1959). The water of the lake has a fairly high salt content (up to 5.8 g/l water) (Matveyev, 1959); which is approximately half as much salt as in waters of the Aral Sea. The predominating ions, Cl (24.1%) and SO_4 (23.8%), make the water undrinkable.

The neighbourhoods of the lake, especially the coastal sandbanks are saturated with the water from the lake and thus they are extremely salinated. This is

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the reason why there is only sporadic vegetation, predominantly lichens, sometimes counterchanged by the other halophile plants (grasses).

During our continuous studies of the fauna and flora of Central Asia we turned our attention to endemic lichen from Central Asia, *Acorospora gobiensis*. The fatty acid content in this, as well as in some other lichens will be described elsewhere. The GC–MS at low resolution exhibited the cleavage of molecular ion caused by isotopic abundance of Br atoms. The $(M–Br)^+$ ions were significant as well. Because of this fact the chemical analysis of the extract was carried out to detect the presence of brominated fatty acids and also the bromoperoxidase activity in the extract from lichen.

More than ten years ago a paper (Plat, Krenn, & Wever, 1987) documented the bromoperoxidase activity in the common lichen *Xanthoria parientia* but no attempts were undertaken to identify the metabolites produced by this enzyme.

The lichen was homogenised and after centrifugation the supernatant was dialysed against Tris sulphate buffer pH 8.0. The sample was then separated by gel electrophoresis (8% polyacrylamide gel, 0.2% SDS). After staining there were many bands, those were screened by atomic absorption for the presence of heavy metal. The only one was positive and the detected heavy metal atom was vanadium. The molecular weight of the native bromoperoxidase (determined by comparison with known standard proteins) was 144 000. The activity of the enzyme (see Section 3) was 14.5 μm of brominated monochlorodimedone min^{-1} counted for 5 g of starting amount of the lichen.

The methyl ester of major acid I had a molecular formula $C_{19}H_{29}O_2Br$, which was established by HR-MS-PCI (high-resolution mass spectrometry with positive chemical ionisation). The infrared spectrum displayed an acetylenic band at 2210 cm^{-1} and an ester group at 1730 cm^{-1}. The UV absorption [λ_{max} were 214 nm (ε, 19 800) and 233 nm (ε, 11 400)] suggested the presence of an en-yne (C=C–C≡C) system. The ^{1}H- and ^{13}C-NMR spectra revealed signals in Table 1. The large vicinal coupling constant (J = 14.0 Hz) between 17-H and 18-H indicated 17*E* geometry. 17-H was further coupled to 14-H_2 by J = 2.2 Hz, which is diagnostic for an en-yne system. Additional olefinic signals at δ 5.34 (dt, 1H, J = 15.5 and 7.0 Hz) and 5.38 (dt, 1H, J = 15.5 Hz) implied the presence of a further *trans* double bond. The ^{1}H-NMR data exhibited also signals for a methoxyl group and seven unresolved methylene groups (from C_7 to C_{13}; 1.47 ppm). ^{13}C-NMR spectrum contained signals indicative of disubstituted acetylene (92.7 and 77.5 singlets). The NMR data for three contiguous methylene (C_2–C_4) protons were identical with literature data (Ichiba et al., 1994; Brantley et al., 1995; Hirsh et al., 1987; Quinn & Tucker, 1985). On the basis of all above described data, we suggest that structure I is a methyl ester of (5*E*,17*E*)-18-bromo-octadeca-5,17-diene-15-ynoic acid.

The methyl ester of the second acid has a molecular formula $C_{19}H_{25}O_2Br$, determined by HR-MS-PCI with NH_3 as ionisation gas. Infrared bands at 2221 and 1740 cm^{-1} suggested acetylenic bonds and an ester group. In the UV spectrum a major peak (λ 225 nm, ε 15800) was shown characteristic for a conjugated en-yne system of unsaturated bonds. The signals from

Table 1. ^{1}H-NMR and ^{13}C-NMR data of brominated fatty acids from Tian-Shan lichens

Carbon No.	**I** ^{1}H-NMR	**II** ^{1}H-NMR	**I** ^{13}C-NMR	**II** ^{13}C-NMR
1	–	–	179.1 s	178.8 s
2	2.32 t (2H, J = 7.5 Hz)	2.39 t (2H, J = 7.3 Hz)	33.9 t	33.1 t
3	1.63 quin (2H, J = 7.5 Hz)	1.88 tt (2H, J = 7.3; 6.7 Hz)	24.2 t	23.3 t
4	1.97 m (2H)	2.33 m (2H)	32.4 t	18.5 t
5	5.38 dt (1H, J = 15.5; 7.4 Hz)	–	130.8 d	79.7 s
6	5.34 dt (1H, J = 15.5; 7.0 Hz)	–	129.5 d	63.1 s
7	1.95 m (2H)	–	32.4 t	68.5 s
8	1.53 m (2H)	–	29.6 t	78.9 s
9	1.47 m (2H)	2.21 tt (2H, J = 6.9; 0.8 Hz)	29.2 t	19.0 t
10	1.47 m (2H)	1.47 m (2H)	29.5 t	27.9 t
11	1.47 m (2H)	1.47 m (2H)	29.3 t	28.7 t
12	1.47 m (2H)	1.47 m (2H)	28.8 t	28.8 t
13	1.47 m (2H)	1.47 m (2H)	28.9 t	28.9 t
14	2.26 ddt (2H, J = 2.2; 6.8; 0.6 Hz)	1.47 m (2H)	19.1 t	28.6 t
15	–	1.61 m (2H)	92.7 s	27.6 t
16	–	2.33 m (2H)	77.5 s	18.5 t
17	6.17 dt (1H, J = 14.0; 2.2 Hz)	–	118.0 d	83.2s
18	6.57 d (1H, 14.0; 0.6 Hz)	–	117.1 d	84.5 s
OMe	3.67 s (3H)	3.65 s (3H)	51.3 q	51.3 q

^{1}H- and ^{13}C-NMR spectra are also included in Table 1. Predominantly CH_2 at 2.22 ppm (C_4) adjacent to an acetylenic bond, which was coupled to a CH_2 (C_3) ($J = 6.7$ Hz) at δ 1.88 and a CH_2 (C_2) triplet ($J = 7.3$ Hz) at 2.39 ppm. Other methylene groups in the ^{1}H-NMR spectrum were at 2.21 ppm (C_9) and a multiplet at 2.37 ppm (C_{16}). The ^{13}C-NMR spectrum showed six signals for triple bonds (as singlets) and none for double bond(s). All above mentioned data are in good agreement with structure II, i.e. methyl ester of 18-bromooctadeca-5,7,17-triynoic acid.

These are the first brominated fatty acids identified from a terrestrial plant. In contrast to brominated fatty acids from marine invertebrates they have a lower number of unsaturated (double and triple) bonds. This may be caused by the higher temperature of the lichen environment. The temperature of the sand and the stones where the lichens usually grow is often as high as 80°C (during sunny days), but the sea water reaches maximally 30°C.

3. Experimental

The total lipid extract (Bligh & Dyer, 1959) as viscous dark oil was eluted from Sephadex LH-20 column with chloroform–hexane 65:35 and then separated by RP-HPLC (Supelco, RP-18, 85% MeOH–H_2O) afforded compounds $\mathbf{I}_a$–$\mathbf{II}_a$ (approximately 13.1 and 8.8 mg, respectively, from ca. 50 g of lichen). The free acids were treated with a solution of CH_2N_2 in diethylether. The crude methyl esters were further purified by RP-HPLC (Supelco, RP-18, 95% MeOH–H_2O).

The brominating activity of the enzyme was determined according to Plat et al. (1987). Briefly, the supernatant was mixed with a mixture containing 0.1 M sodium acetate buffer, pH 5.5, 0.1 mM KBr, 50 μM monochlorodimedone and 1 mM H_2O_2. The activity was expressed as μM of monochlorodimedone brominated/min (the decline of absorbance at 290 nm from $\varepsilon = 20.2$ mM^{-1} monochlorodimedone to monochlorobromodimedone $\varepsilon = 0.2$ mM^{-1}). Sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis was carried out as described previously (Rezanka et al., 1992) by using 8% polyacrylamide gels ($90 \times 180 \times 1$ mm) containing 0.2% SDS. The gels were stained for protein identification using Coomassie Blue R-250. The colour bands were transported into AAS. The molar masses of the proteins were estimated from the relative mobilities of standards (Sigmamarkers-high range kit, M.W. 36 000–205 000).

UV spectra were measured by the Cary 118 (Varian) apparatus in heptane within the range of 200–350 nm. For scanning infrared spectroscopy of methyl esters as neat film a Perkin-Elmer Model 1310 (Perkin-Elmer, Norwalk, CT) infrared spectrophotometer was used. NMR spectra were recorded on a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe) at 500.1 MHz (^{1}H) and 125.7 MHz (^{13}C). High- and also low-resolution mass spectra were recorded using a VG 7070E-HF spectrometer (positive chemical ionisation mode with NH_3, source temperature 130°C). Ionising conditions for CI was 70 eV. The V content of the enzyme was determined with an AAnalyst 100, atomic absorption spectrometer (Perkin-Elmer).

Methyl ester of 18-bromo-(5E,17E)-octadeca-5,17-diene-15-ynoic acid (**I**) UV λ_{max} (MeOH, nm) 214 (ε 19 800), 233 (ε 11 400); IR (as neat film) 2810, 2210, 1730, 1635, 1560, 1440 and 915 cm^{-1}; MS-CI (NH_3, m/z) $(M + NH_4)^+$ 386 and 388; HR-MS-PCI (NH_3, m/z) calcd for $(C_{19}H_{29}O_2Br + NH_4)^+$ 386.4771 and 388.4771, found 386.4769 and 386.4773 (intensity ratio 1:0.97).

Methyl ester of 18-bromooctadeca-5,7,17-triynoic acid (**II**) UV λ_{max} (MeOH, nm) 225 (ε 15 800); IR (as neat film) 2221, 1740, 1608 and 1445 cm^{-1}; MS-CI (NH_3, m/z) $(M + NH_4)^+$ 382 and 384; HR-MS-PCI (NH_3, m/z) calcd for $(C_{19}H_{25}O_2Br + NH_4)^+$ 382.4461 and 384.4461, found 382.4457 and 384.4453 (intensity ratio 1:0.98).

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